

Transforming Growth Factor β 1 Is Expressed in the Jejunum after Experimental *Cryptosporidium parvum* Infection in Humans

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Biopsies from volunteers challenged with *Cryptosporidium parvum* were examined for transforming growth factor β 1 (TGF- β 1). None of the prechallenge biopsies exhibited TGF- β . Seven of 12 volunteers with oocyst shedding expressed TGF- β versus 2 of 13 volunteers without detected oocysts. The association of TGF- β expression with oocyst excretion and the timing of symptoms suggests that TGF- β mediates intestinal healing.

Transforming growth factor β (TGF- β) is an anti-inflammatory cytokine that stimulates repair of damaged mucosal epithelial integrity following injury (9, 20). Lymphocytes secreting TGF- β downregulate host immune and inflammatory responses, especially in the intestinal mucosa (3, 13, 24). Absence of these regulatory lymphocytes is thought to play an important role in the pathogenesis of inflammatory bowel diseases (1, 3, 13, 25, 27).

TGF- β is also a key signal for epithelial repair in vitro (4, 20). TGF- β stimulates restitution by causing migration of epithelial cells into denuded areas, deposition of extracellular matrix, and restoration of epithelial barrier integrity (1, 4). TGF- β has the striking ability to repair the permeability defect of intestinal monolayers induced in vitro by *Cryptosporidium parvum* infection or gamma interferon (IFN- γ) (18, 19). We reasoned that in cryptosporidiosis, following epithelial cell damage and barrier integrity disruption by *C. parvum* and inflammatory cytokines, TGF- β may be needed to restore epithelial integrity and promote healing.

The studies described here were approved by the committee for the protection of human subjects at the University of Texas at Houston and the Institutional Review Board for Human Subjects at Baylor College of Medicine. Volunteers were orally challenged with defined doses of *C. parvum* oocysts as part of ongoing studies aimed at determining the infectious dose (2, 5, 15–17). Assays of stools for oocyst excretion, prechallenge anti-*C. parvum* immunoglobulin G measurement, symptom recording, and collection of intestinal biopsies were performed as previously described (5, 6). Biopsy specimens were immediately treated with paraformaldehyde in diethylpyrocarbonate-treated water. Plasmids containing human TGF- β 1 cDNA (American Type Culture Collection, Manassas, Va.) and ³⁵S-labeled riboprobes were prepared using methods previously described (22, 26). Sections were probed by in situ hybridization and graded as previously described (22, 26).

Twenty-nine immunocompetent volunteers were experimentally infected with *C. parvum*. Ten volunteers had prechallenge endoscopies, and 35 separate endoscopies were performed on 27 volunteers postchallenge. Eleven volunteers were seropositive for anti-*Cryptosporidium* immunoglobulin G before challenge, as determined by enzyme-linked immunosorbent assay.

Eighteen volunteers were seronegative, including three for whom only prechallenge biopsies were available for this study.

We detected TGF- β mRNA in postchallenge biopsies from 9 of 27 volunteers. TGF- β mRNA was detected as numerous silver granules overlaying cells in the epithelium of crypts and villi (Fig. 1). None of the prechallenge biopsies exhibited TGF- β expression. None of the nine biopsies collected 1 to 4 days postchallenge exhibited TGF- β expression. In contrast, 3 of 13 biopsies from days 5 to 13 postchallenge and 6 of 14 from day 14 onward exhibited TGF- β mRNA expression. Since TGF- β expression was exhibited only in biopsies obtained \geq 5 days postchallenge, we excluded from subsequent analyses two patients who had biopsies only during the first few days postchallenge. TGF- β was expressed equally in seropositive (4 of 11, or 36%) and seronegative (5 of 14, or 36%) volunteers (Table 1).

None of the biopsies from four asymptomatic volunteers exhibited TGF- β expression. In contrast, 9 of 21 symptomatic volunteers expressed TGF- β ($P = 0.26$, Fisher's exact test) (Table 1). Thus, while TGF- β was expressed only in volunteers with symptoms, this association was not statistically significant. Among biopsies collected >5 days postchallenge, TGF- β was expressed in 7 of 12 volunteers with oocyst shedding. By contrast, only 2 of 13 volunteers who did not shed oocysts expressed TGF- β ($P < 0.05$, Fisher's exact test) (Table 1).

When symptoms and oocyst shedding were used together as measures of injury, three groups with progressively more evidence of injury were defined: those with neither symptoms nor oocyst shedding, those with symptoms without oocyst shedding, and those with both symptoms and oocyst shedding, and 0 of 4, 2 of 9 (22%), and 7 of 12 (58%), respectively, expressed TGF- β mRNA. Thus, there is a direct correlation between clinical evidence of injury and TGF- β expression.

Since healing and repair should follow injury, the timing of signals for healing should be similar to that of symptoms and subsequent healing. TGF- β was expressed in only 1 of 11 biopsies collected before onset of symptoms. In contrast, 4 of 8 biopsies collected from volunteers during symptoms and 4 of 10 biopsies collected from volunteers following resolution of symptoms exhibited TGF- β expression.

Most of the volunteers who developed symptoms did so between 6 and 13 days postchallenge. Among symptomatic volunteers, significantly more of those who shed oocysts expressed TGF- β in biopsies obtained at <14 days postchallenge (3 of 6 versus 0 of 10, $P < 0.04$, Fisher's exact test). In contrast, the proportion expressing TGF- β at ≥ 14 days was similar (Fig.

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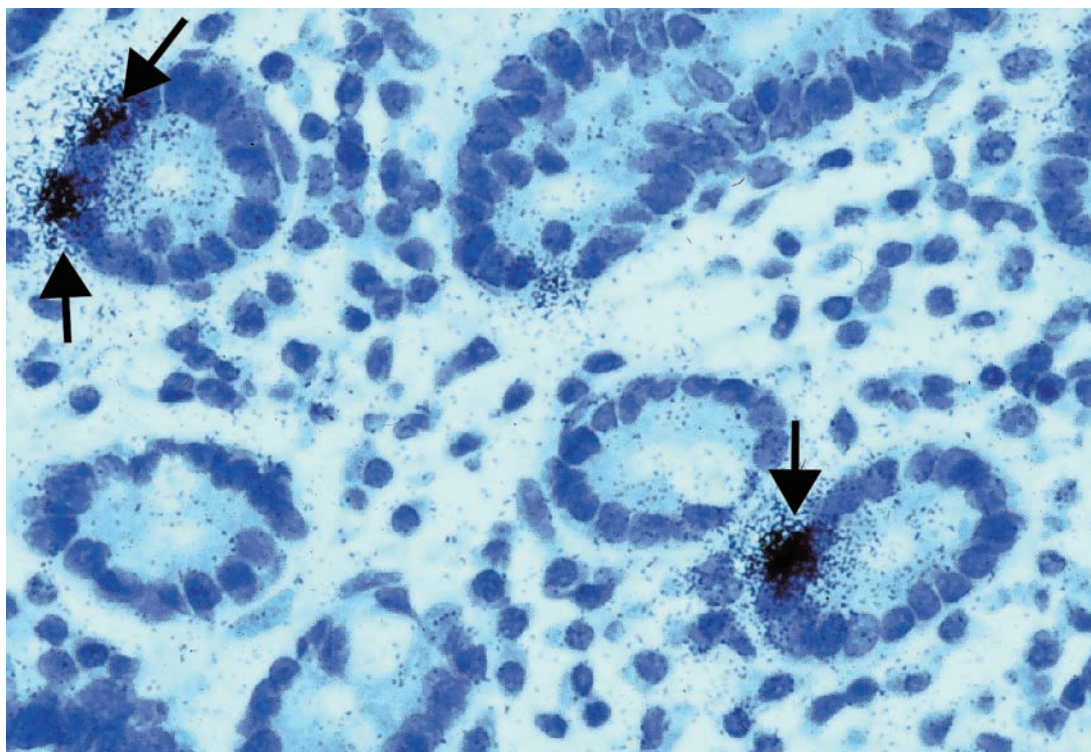


FIG. 1. Expression of TGF- β mRNA in jejunal biopsies from healthy volunteers experimentally challenged with *C. parvum*. A jejunal biopsy from a healthy volunteer with experimental human cryptosporidiosis was probed by in situ hybridization using 35 S-labeled riboprobes for TGF- β 1. Cells within the crypt epithelium expressing TGF- β mRNA are overlaid with numerous black silver grains (arrows). Magnification, $\times 400$.

2). Thus, TGF- β expression began during the period of symptoms in volunteers with both symptoms and oocyst shedding, but only during later phases in those without oocyst shedding.

In this study, we have demonstrated TGF- β expression within the intestinal epithelium after experimental human *C. parvum* infection. TGF- β expression correlated directly with oocyst shedding, a measure of parasite burden. Furthermore, patients with both symptoms and oocyst shedding were more

likely to express TGF- β than those with just symptoms. The development of symptoms likely reflects the level of epithelial injury and/or dysfunction. Similarly, parasite burden correlates with the number of epithelial cells infected and associated injury and permeability changes (7). The timing of TGF- β expression corresponded to the waning of symptoms and the beginning of the healing stage.

TGF- β is known to play an important role in normal intestinal physiology. Mice with a disrupted TGF- β gene die of multifocal inflammatory disease, which includes gastrointestinal tract involvement (23). Inadequate expression of TGF- β is thought to be associated with the pathogenesis of chronic

TABLE 1. Correlation of expression of TGF- β mRNA with symptoms, oocyst excretion, and prechallenge anti-*Cryptosporidium* antibody

Factor	No. of volunteers with TGF- β /total (%) ^a
Prechallenge anti- <i>C. parvum</i> antibody	
Present.....	4/11 (36)
Absent	5/14 (36)
Gastrointestinal symptoms ^b	
Present.....	9/21 (43)
Absent	0/4 (0)
Oocyst excretion	
Present.....	7/12 (58) ^c
Absent	2/13 (15)

^a Postchallenge biopsy samples from 27 volunteers were probed for TGF- β ; 2 volunteers who had biopsies only prior to day 5 postchallenge were excluded from these analyses.

^b Symptoms include diarrhea, abdominal pain, and nausea or vomiting.

^c $P < 0.05$ for those excreting measurable numbers of oocysts compared to those without detectable numbers of oocysts.

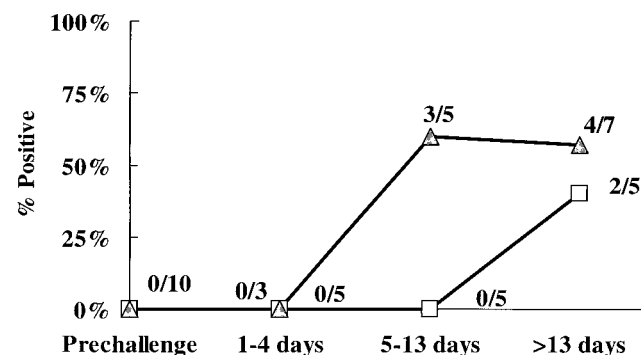


FIG. 2. Timing of TGF- β expression in symptomatic volunteers with or without oocyst excretion. The proportions of volunteers with TGF- β expression as seen by in situ hybridization were determined for samples obtained prior to challenge or at 1 to 4, 5 to 13, or ≥ 14 days postchallenge in symptomatic volunteers with (triangles) and without (squares) oocyst excretion.

bowel inflammation in immunodeficient mice (1, 3, 8, 12, 21, 25). In these models, intestinal injury is mediated by type 1 cytokines and can be reversed by cells expressing TGF- β . Similarly, in murine toxoplasmosis, overexpression of IFN- γ early without coexpression of TGF- β leads to fatal intestinal necrosis (10, 11, 28). Mice survive if interleukin-10 is produced, which likely acts by stimulating TGF- β production (14, 28; L. H. Kasper, H. Debbabi, A. C. Lepage, J. D. Scharzmann, and D. Buzoni-Gatel, *Innate Acquir. Immun. Mucosal Surfaces*, Keystone Symp., abstr. 113, 2000). Thus, TGF- β controls the inflammatory response and allows intestinal healing following injury induction by type 1 cytokines. Interestingly, out of the nine volunteers who expressed TGF- β , seven had also expressed proinflammatory cytokines (either tumor necrosis factor alpha, interleukin-15, or IFN- γ) (data not shown).

In summary, TGF- β was expressed in jejunal biopsies of volunteers experimentally challenged with *C. parvum*. TGF- β was expressed during the symptomatic and resolution phases of infection and was significantly associated with markers of injury. TGF- β is expressed earlier in volunteers shedding oocysts than in those who do not shed oocysts. Taken together, these data suggest that TGF- β plays the role of an anti-inflammatory cytokine involved in healing and restitution in cryptosporidiosis.

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